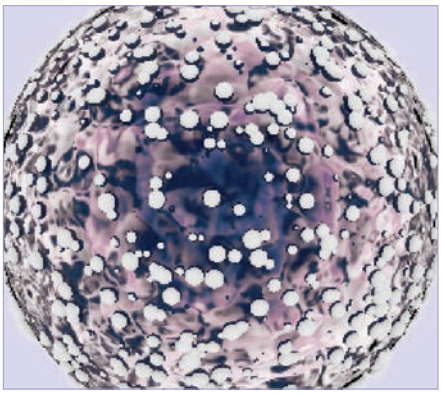

Prognostic Biomarkers in Melanoma: Tailoring Treatments to the Patient

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ABSTRACT

BACKGROUND: it is often difficult to accurately predict how a melanoma will progress because melanomas can be so diverse in their genetic and histological makeup. **OBJECTIVE:** We sought to characterize the current state and progression of biomedical markers towards their utilization as prognostic indicators for patients with melanoma. **METHODS:** A literature search of the research repository databases PubMed and GoogleScholar was conducted using the following inclusion criteria: (1) published within the last 10 years, and (2) use of overall survival, disease progression, or clinical outcome as primary endpoints. Search terms included various permutations of "biomarkers," "prognostic," "immunologic," "serologic," "visual," and "melanoma." Results were evaluated for statistical power, results significance, and experimental design integrity. **RESULTS:** The prognostic capabilities of clinical tests for malignant melanoma have made great strides in the last few years, with several serologic and immunohistochemical biomarkers being preliminarily linked to various measures of clinical prognosis. While clinical feasibility of a single sensitive and specific biomarker remains unfeasible, use of select combinations of tested biomarkers remain viable. **CONCLUSION:** Diagnostic and prognostic genetic assays have begun to cross over from research to commercial application, giving physicians additional tools during the early stages of diagnosis to optimize and individualize treatments.

KEY WORDS: Immunologic marker, serum marker, screening assay, checkpoint inhibitor, melanoma

Because melanomas can be so diverse in their genetic and histological makeup, it is often difficult to accurately predict how a melanoma will progress. Yet, accurate assessment of this process, including estimating metastatic risk at early tumor stages, is an important factor in reducing patient mortality. Malignant melanomas remain the deadliest form of skin cancer, responsible for an estimated 9,320 deaths in 2018.[1] While factors such as age, ethnicity, and tumor location[2] can discern broad trends on a macro level, they are not sensitive enough for individual cases. Current standards used as prognostic factors include mitotic rate, Breslow death, and sentinel lymph node (SLN) assessment.[3] The American Joint Commission on Cancer (AJCC) includes these variables to stage and classify tumors and generate survival curves for each stage, allowing for more accurate clinical decision-making. However, even in the most recent eighth edition of the AJCC staging system, the lack of novel prognostic biomarkers is strikingly apparent.[3,4]

In the era of genomics, the use of large-scale screening assays to screen for a predetermined set of markers has largely rendered the idea of a single, tell-all biomarker an archaic notion. Genetic tests are already commercially available to aid clinicians with melanoma staging and diagnosis. Treatment options for melanoma have also evolved, with checkpoint inhibitors improving patient survival rates[5] for late-stage melanoma. However, therapeutic benefits vary between individuals, as studies have expressed difficulty in anticipating patient responses[6] as well as the insufficiency of conventional response criteria for predicting therapeutic benefit.[7] This variation has driven the search for biomarkers which can serve as indicators of patient response to therapy. Recently, a number of studies have been able to uncover several genetic and protein markers that show statistically significant associations with mortality, improved clinical outcome, and melanoma progression. We aim to summarize and highlight these markers, which have the potential to aid in the development of individualized treatments for malignant melanomas in the future.

METHODS

A literature search of the research repository databases PubMed and GoogleScholar was conducted using the following inclusion criteria: (1) published within the last 10 years,and (2) use of overall survival, disease progression, or clinical outcome as primary endpoints. Search terms included various permutations of "biomarkers," "prognostic," "immunologic," "serologic," "visual," and "melanoma." Results were evaluated for statistical power, results significance, and experimental design integrity.

RESULTS

Immunohistochemical markers. The wave of retrospective reviews in the last five years has brought up many immunohistochemical biomarkers associated with the prognosis of malignant melanomas, many of which are summarized in Table 1. It is important to stress

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that many of these studies were limited by having small cohorts, using single centers, or studying rare variants of malignant melanoma. These initial findings will need to be replicated and expanded for any definitive claims to be made; however, they add to the pool of potential markers and therapeutic targets to be tested for future use.

Adenylate cyclase–associated protein (CAP2). CAP2 is an actin monomer binding protein that plays a regulatory role in actin assembly and disassembly in the cell.[8] It has been shown to play roles in the proliferation and migration of breast cancer cells and is overexpressed in hepatocellular carcinoma.[9] More recently, a retrospective study of 50 patients with metastatic melanoma found that CAP2 overexpression was significantly associated with increased tumor thickness and poorer clinical outcomes. In addition, gene expression increased in a stepwise pattern as melanoma stage advanced, which opens the possibility of it being a marker of cancer progression.[8]

Epidermal growth factor receptor (EGFR). The EGFR family, consisting of EGFR/HER1, HER2, HER3, and HER4, regulates several cellular processes important for growth and survival, including cell division, apoptosis, and migration.[10] HER4 expression is normally associated with antiproliferative and pro-apoptotic activity. It is also frequently mutated in malignant melanomas (19%), raising the question of whether it could be used to inform patient prognosis or identify malignancies earlier in the clinical setting. In two separate single-center retrospective studies, HER4 expression levels were found to be significantly associated with patient survival and prognosis.[10,11] While HER4 can have both oncogenic and tumor-suppressor properties, both studies found HER4 expression to be oncogenic in malignant melanoma, with high expression levels significantly associated with shorter overall progression-free survival.[10,11] Patients positive for HER4 expression were also shown to have lower overall survival than those who were HER4-negative.[10,11] These studies point to HER4 as a potential predictor of patient prognosis or at least a molecular therapeutic target.

Cancerous inhibitor of protein phosphate 2a (CIP2A). CIP2A normally regulates C-myc and AKT through its inhibition of protein phosphatase 2A, but it is also an oncogene that promotes tumor transformation and cancer progression.[12] CIP2A overexpression has been noted in chronic myelogenous leukemia, ovarian cancer, prostate cancer, and colon cancers, among others.[12,13] It has also been recommended as a therapeutic target for head and neck squamous cell carcinoma and triple negative breast cancer.[13] Recently, CIP2A was also shown to be overexpressed in malignant melanomas, with high expression significantly associated with poor patient survival and Breslow thickness. Interestingly, there is a distinction between nuclear overexpression of CIP2A, which is associated with poorer overall survival, and cytoplasmic overexpression of CIP2A, which was reported to be correlated with longer relapse-free survival.[12] While further research needs to be conducted, these results at least corroborate the idea that CIP2A levels could be used as a predictor of overall survival in melanomas.

Aldehyde dehydrogenases (ALDHs). ALDHs play an important role in tumor cells, neutralizing aldehydes by converting them to carboxylic acids to prevent toxic buildup.[14] Previous research has shown that an overexpression of ALDH variants is associated with tumorigenic and chemotherapy-resistant melanoma cells and that ALDH activity is epigenetically upregulated in melanoma cells.[15] In addition, it has been reported that silencing of the ALDH1A isozyme leads to cell cycle arrest, reduced tumorigenesis, and drug-sensitized melanoma cells.[15] It is interesting then that high levels of ALDH1 activity are associated with better patient outcomes, including decreased rates of melanoma-specific death.[16] This is not the only cancer with this inverse relationship, and the variability might be caused by the many different roles ALDH1 could play in each tumor.

Serum markers. Serologic markers are extremely appealing as biomarker candidates as testing is less invasive and has relatively fast turnaround times.[17,18] The identification of new metabolites, antigens, and enzymes that could be used as a marker of disease progression and predictors of patient outcomes has driven research over the last few years. Another focus of research has been the re-evaluation of classic biomarkers for their clinical utility and prognostic strength.

RNAs. MicroRNAs (miRNAs) are responsible for attenuating gene expression post-translationally and play many roles in cellular processes, including immune response, apoptosis, and proliferation. They have also been implicated in tumorigenesis and metastasis.[19,20] Recently, several serum miRNAs have been reported to be associated with cancer progression and patient prognosis, including miRNA 20621, miR-15022, miR-42522, and miR-1623. When used in an assay, it was reported that a certain miRNA signature was predictive of melanoma recurrence—a finding that could aid in the clinical management of patients at the time of diagnosis.[22] Factors implicated in mRNA regulation, such as translation initiation factor 4E (eIF4E), have also been associated with reduced survival.[49] Because of the stability of blood and the standardization of assays, blood-based miRNA assays may be low-cost, effective prognostic predictors for malignant melanomas in the future.

5-S-cysteinyl-dopa (5-S-CD) and lactate dehydrogenase (LDH). RNA and genetic markers are not the only category of markers being evaluated for their prognostic capability. 5-S-CD and LDH are a metabolite and enzyme routinely used as serum biomarkers in Japan.[24] In one large-scale retrospective review, it was suggested that 5-S-CD levels could significantly distinguish between patients with good and poor prognoses and that elevated levels reflect disease progression.[24] LDH has been suggested as a prognostic indicator for some time, but results have been controversial. A recent meta-analysis attempted to further clarify the relationship between LDH and melanoma and found that the high LDH levels could be a predictor of poor prognosis among melanoma patients.[25]

Biomarker applications in malignant melanoma treatments. Recently, the number of treatments for malignant melanoma has expanded. Immune checkpoint inhibitors, which harness T-cells to amplify immune system responses against tumors, have been extensively researched due to promising long-term clinical results. However, obstacles such as developed resistance and limited treatment scope make traditional chemotherapy the main option for many patients.

Programmed death ligand 1 (PD-L1). PD-L1 is a ligand which binds to programmed death 1 (PD-1) immunoglobin to suppress T-cell activity.[26–29] The ligand is expressed in both hematopoietic and nonhematopoietic tissues as

TABLE 1. Prognostic biomarkers of malignant melanoma

BIOMARKER	GENE	ASSOCIATIONS
Adenylyl cyclase–associated protein 2[8]	CAP2	Higher levels of expression were associated with poorer clinical outcomes
CD169+ cells[39]	–	High numbers of cells were correlated with favorable overall survival
Human epidermal growth factor receptor[11]	HER4	Higher levels of expression were associated with shorter duration of progression-free survival
Cancerous inhibitor of pro-tein phosphate 2a[12]	CIP 2A	Higher levels of expression were associated with poorer clinical outcomes
Soluble CD73[40]	CD73	Higher levels of soluble CD73 enzyme associated with poorer clinical outcomes
Melanocortin receptor 1[41]	MCR1	Presence of any variant of MCR1 was associated with poorer clinical outcomes
S100B[42]	S100B	Higher levels of expression were associated with poorer clinical outcomes
Aldehyde dehydrogenase 1[15, 6]	ALDH1	Higher levels of expression were associated with better prognosis
MicroRNA 16[23]	miR-16	Decreased serum levels were associated with advancing melanoma stage
MicroRNA 15[22]	miR-15b, miR-425	Increased serum levels measured prior to melanoma recurrence
MicroRNA 206[21]	Mir-206	Decreased serum levels are associated with poor clinical prognosis
MicroRNA 4633-5p[43]	miR-4633-5p	Differentially higher expression was associated with better clinical outcomes
MicroRNA 330-5p[44]	miR-330-5P	Increased exosome concentration was associated with melanoma presence
MicroRNA 10b	miR-10b	Increased expression associated with metastasis
BRCA-associated protein 1[41,45]	BAP1 (piris too)	Deficient BAP1 expression was associated with decreased survival
Glucose-regulated protein of 78 kD[46]	BiP, GRP78	Increased expression of BiP/GRP78 with poor survival
Melanoma cell-adhesion molecule[47]	MCAM/MUC18	Increasing MCAM/MUC18 intensity correlated with cancer progression
β2-adrenergic receptor[48]	β2AR	Increased expression was associated with poorer clinical outcomes
Melanoma Inhibitory activity[44]	MIA	Higher serum levels were associated with lower overall survival rates
Krüppel-like factor[49]	KLF6	Higher protein levels were associated with lower three-year survival rate
Eukaryotic translation initiation factor 4E[36]	eiF-4E	Increased expressions of eIF4E and phospho-eIF4E were associated with reduced survival and increased risk of death
class III β-tubulin[51]	TUBB3	Decreased expression levels were associated with decreased overall survival and lower progression-free survival
D-dimers[52]	–	Increased plasma levels were associated with poor overall disease outcome

well as in tumor cells. It is thought to help tumor cells evade T-cell–stimulated immunological responses; in fact, PD-L1 expression in desmoplastic melanoma is associated with tumor progression.[27] Anti–PD-1 inhibitors, such as nivolumab and pembrolizumab, have been shown to have antitumoral responses for many cancers, but the need for indicators of progression remains a focus of research.[29] It was recently reported that elevated serum ecto-5′-nucleotidase (CD73) levels were significantly associated with poorer clinical outcomes among patients receiving the PD-L1 inhibitor nivolumab.[28,29] PD-L1 itself has also been tested as a marker for patient progression during immune checkpoint inhibitor therapy, and there have been reported associations between higher starting levels of PD-L1 and worse clinical outcomes.[26] PDL expression levels have also been used to compare the effectiveness of therapies, which could be valuable in the future as a patient-screening method to individualize treatment options.

Melanocortin 1 receptor (MC1R) and β2-adrenergic receptor (β2AR). The presence of MC1R,[47] a G protein–coupled receptor that plays a role in skin pigmentation, was associated with poorer outcomes in patients. The serological biomarker S100B has been one of the most analyzed prognostic indicators, but its clinical application is limited due to its false-positive rate. Contrastingly, β2AR[48] is another G protein–coupled receptor found to be an independent prognostic factor predicting poorer survival and was associated with tumor thickness, ulceration, disease stage, and cell proliferation.

Other serological biomarkers. The tumor-suppressor genes *BRAFV600E* and BRCA-associated protein 1 (*BAP1*) have also been found in combination to be a consistent immunohistologic signature of a specific type of *BAP1* melanocytic lesions. The glucose-regulated protein of 78kd (GRP 78) was also found by Ishikawa et al[46] to be an independent prognostic factor for poor survival; however, its elevation is not specific to malignant melanoma. The melanoma cell adhesion molecule was known to be associated with relapse in melanoma. In a modest 175-patient study, Rapanotti et al[47] found it was a marker of progression even in early stages of melanoma.

Chemotherapies. Oxaliplatin chemotherapy is an option for colorectal cancer that cross-links in DNA to inhibit replication and transcription, and advances are still being made in what it can treat. It was recently reported that XPF protein levels could be used as a patient screening option for melanoma sensitivity to oxaliplatin therapy.[30] Even as novel malignant melanoma treatments are developed, biomarkers still play a role in making traditional therapies more specific to the patients they are most effective for. Another melanoma drug currently in clinical trials, sunitinib, recently had a biomarker analysis performed. Although a couple of candidates (VEGFR1 and P1GF) had correlations with overall survival, no significant associations were made in the study.[31] Predictive biomarkers for the use of patient selection for maximal therapeutic benefit from a treatment are still an extremely important topic of research for the future.

Biomarkers and screening assays. Biomarker assays and assessments that integrate multiple prognostic factors are an aspect of research focusing on combining and weighting current data to make the most accurate predictions concerning malignant melanoma diagnoses and prognoses.[2,18] Because melanomas often have different combinations of mutations and different levels of expression changes, these assays may be able to somewhat make up for skews in small patient cohorts and prove more consistent than tests based on a single biomarker.

Large-scale genomic sequencing assays are also technologically feasible and can be used on large tissue collections and databases to detect biomarker candidates and genetic variants of melanomas, adding to the available information for clinical use. The most available example of this is Decisiondx (Castle Biosciences; Friendswood, Texas), a commercially available 31-gene expression profiling test, which uses 28 discriminating genes and three control genes to analyze a fixed tissue sample of the tumor.[32] Multiple studies[33,34] have supported its prognostic utility in aiding in early-stage identification of patients with a higher risk of relapse.

Other biomarker panels with novel genetic candidates are also in the process of being developed. A recent study by Vendittelli et al[35] analyzed several biomarker candidates from patient serum samples using quantitative polymerase chain reaction. The analysis was able to compare many biomarkers simultaneously and single out one which had expression levels correlated with Breslow thickness and diagnostic capacity.[35,36] Another study used a next-generation sequencing (NGS) panel to sequence several melanoma samples, among other cancers, for somatic variations. Researchers were able to find common expression patterns among the variants, with NGS providing high sequencing fidelity and coverage.[37] Panels screening multiple melanoma microRNAs have been conducted as well.[38] Other research has pointed to using combined bisulfite restriction analysis assays to identify epigenetic markers in malignant melanoma.[39] Overall, improvements in analysis and assay technology have allowed for entire panels of tissue to be analyzed for markers, giving researchers a powerful way by which to study large patient cohorts in the future.

CONCLUSION

The prognostic capabilities of clinical tests for malignant melanoma have made great strides over the last few years. Diagnostic and prognostic genetic assays have begun to cross over from research to commercial application, giving physicians additional tools during the early stages of diagnosis to optimize and individualize treatments. A significant number of biomarkers from many sources remain to be analyzed and tested, with the potential to improve upon and further optimize current tests. As novel treatments for melanoma continue to be developed, innovation must continue to isolate biomarkers which can help track their efficacy.

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